

# Supplementary Materials

## **Cell lysis directed by SulA in response to DNA damage in *Escherichia coli***

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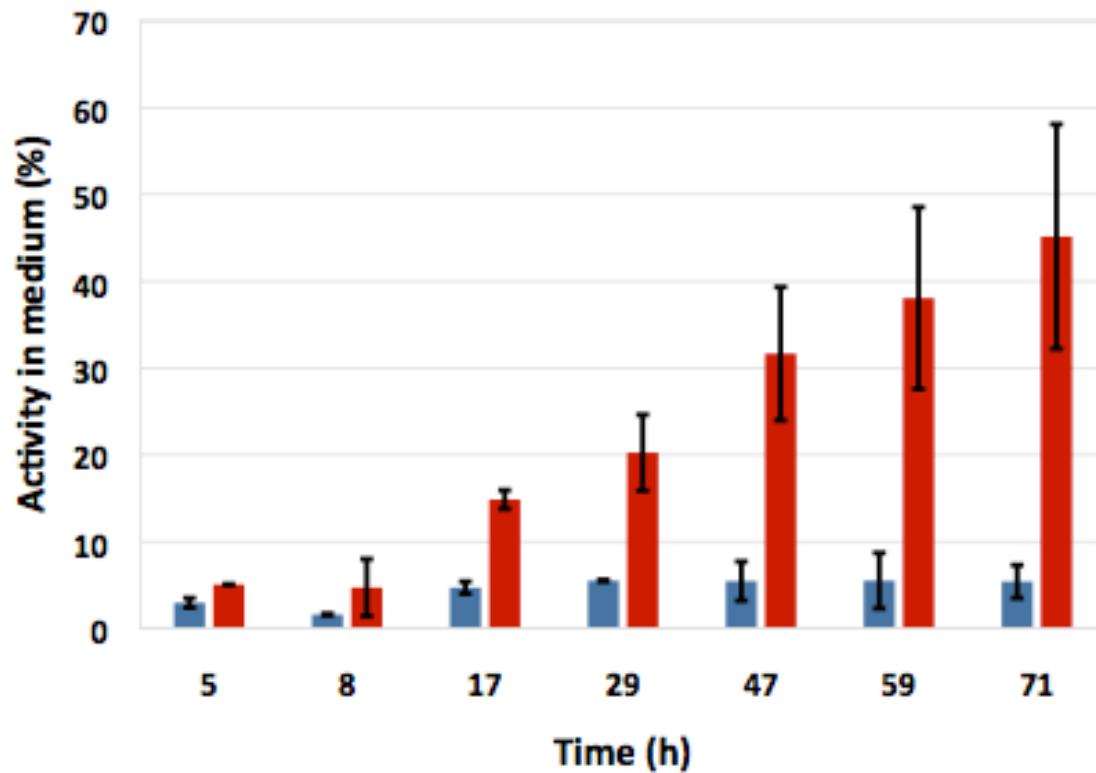
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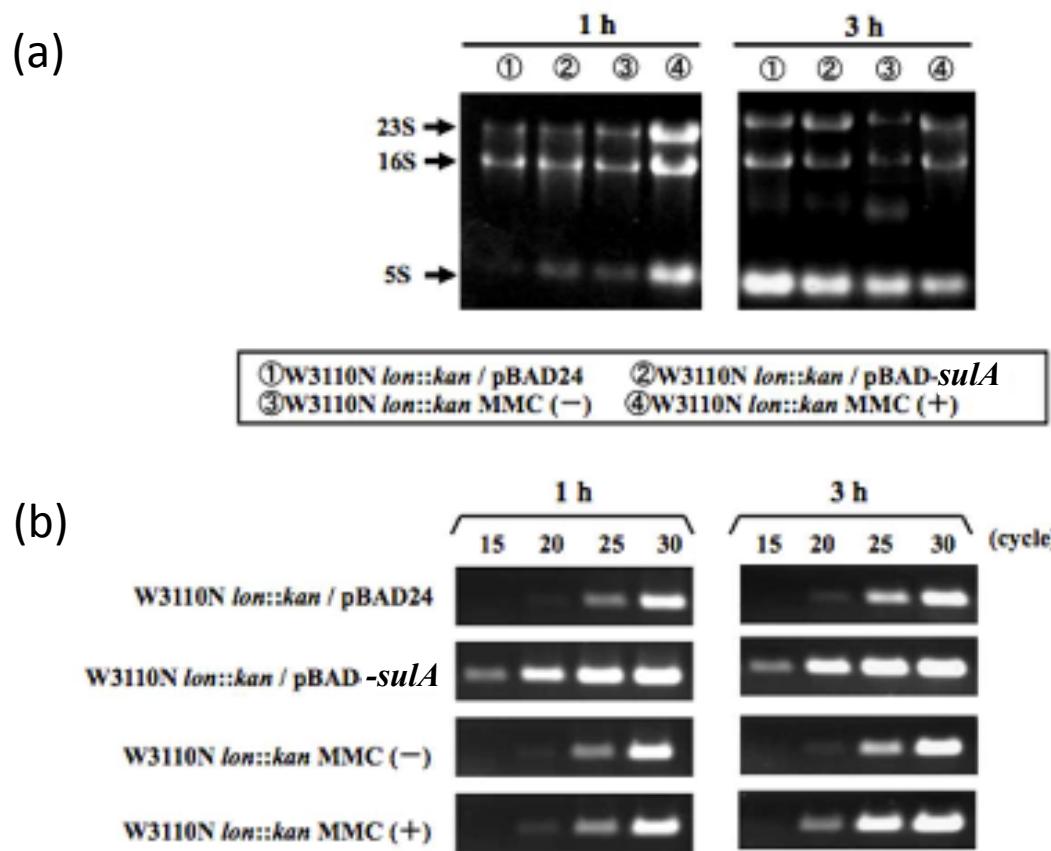
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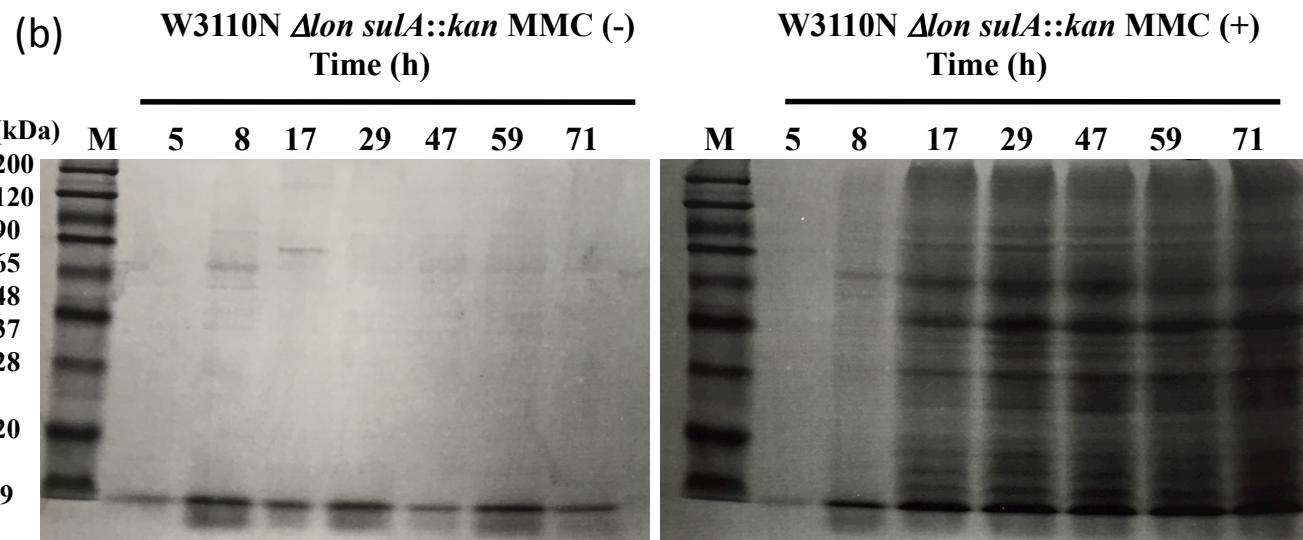
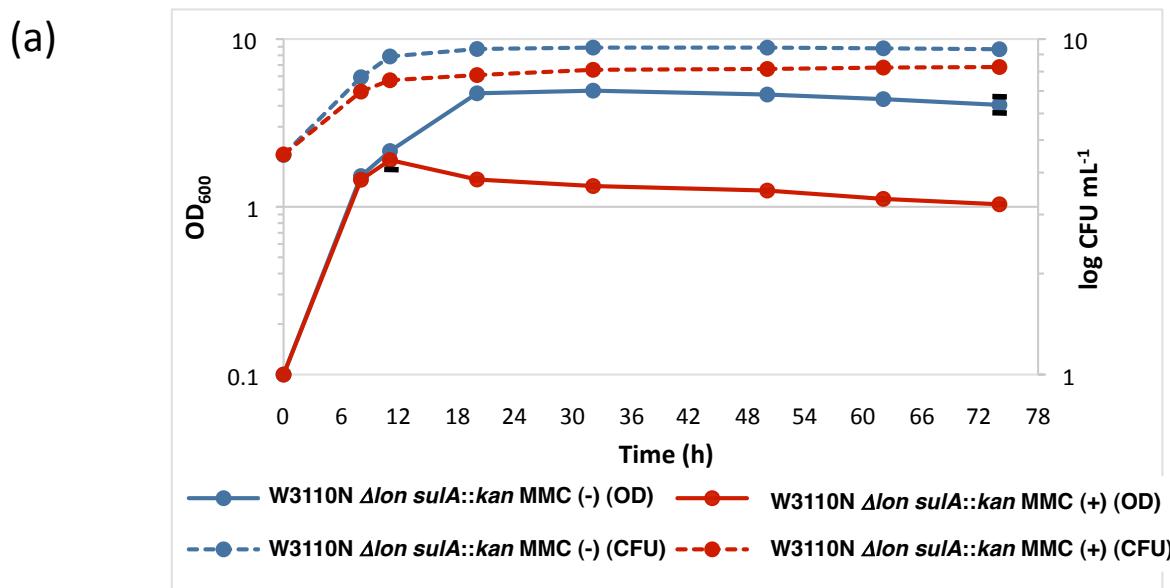
**Figure S1.**  $\beta$ -Galactosidase activity in the medium fraction.

W3110N *lon*::*kan* cells harboring pBAD24 or pBAD-suA were grown in LB medium containing ampicillin and kanamycin. L-arabinose was added at 5 h ( $OD_{600}$  of about 0.5) at the final concentration of 0.1%. Samples were taken at times shown after the addition of arabinose, separated into medium and cell fractions and  $\beta$ -galactosidase activities in both fractions were measured as described in Materials and methods. The activity of the medium fraction is expressed as the percentage of total activity. Open columns and gray columns are W3110N *lon*::*kan* cells harboring pBAD24 and W3110N *lon*::*kan* cells harboring pBAD-suA, respectively. Error bars are  $\pm$ SD.

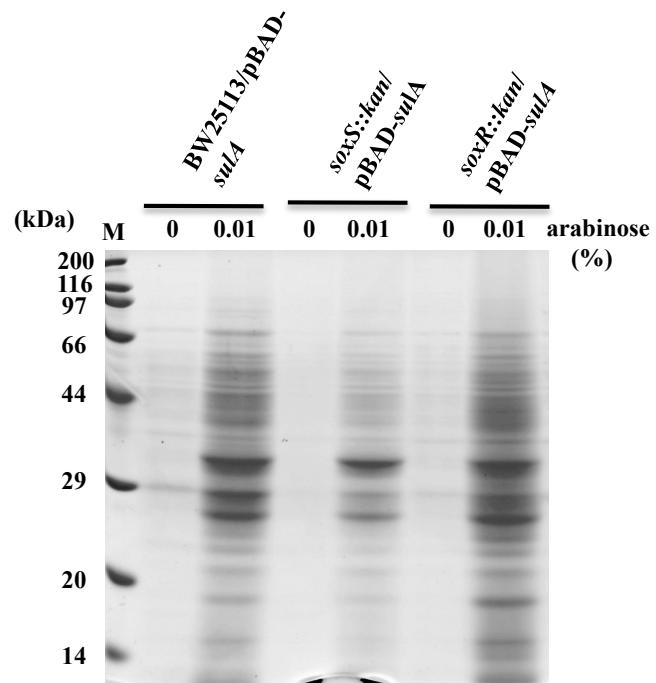


**Figure S2** Expression level of *sulA* under the condition with MMC or with overexpression of *sulA*.

W3110N *lon::kan* cells harboring pBAD24 or pBAD-*sulA* were grown in LB medium containing ampicillin and kanamycin. W3110N *lon::kan* cells were grown in LB medium containing kanamycin. L-Arabinose and MMC were added at OD<sub>600</sub> of about 0.5 at the final concentrations of 0.1% and 0.1 µg/ml, respectively. Total RNA was then isolated from cells at 1 h and 3 h after the induction and subjected to RT-PCR as described in Materials and methods. (a) Total RNA (10 µg) was run and separated by 1.2% agarose gel electrophoresis. (b) RT-PCR was performed with specific primers for *sulA*. After RT reaction, PCR reaction was performed, and the products obtained every 5 cycles from 15 to 30 cycles were analyzed by 0.9% agarose gel electrophoresis.

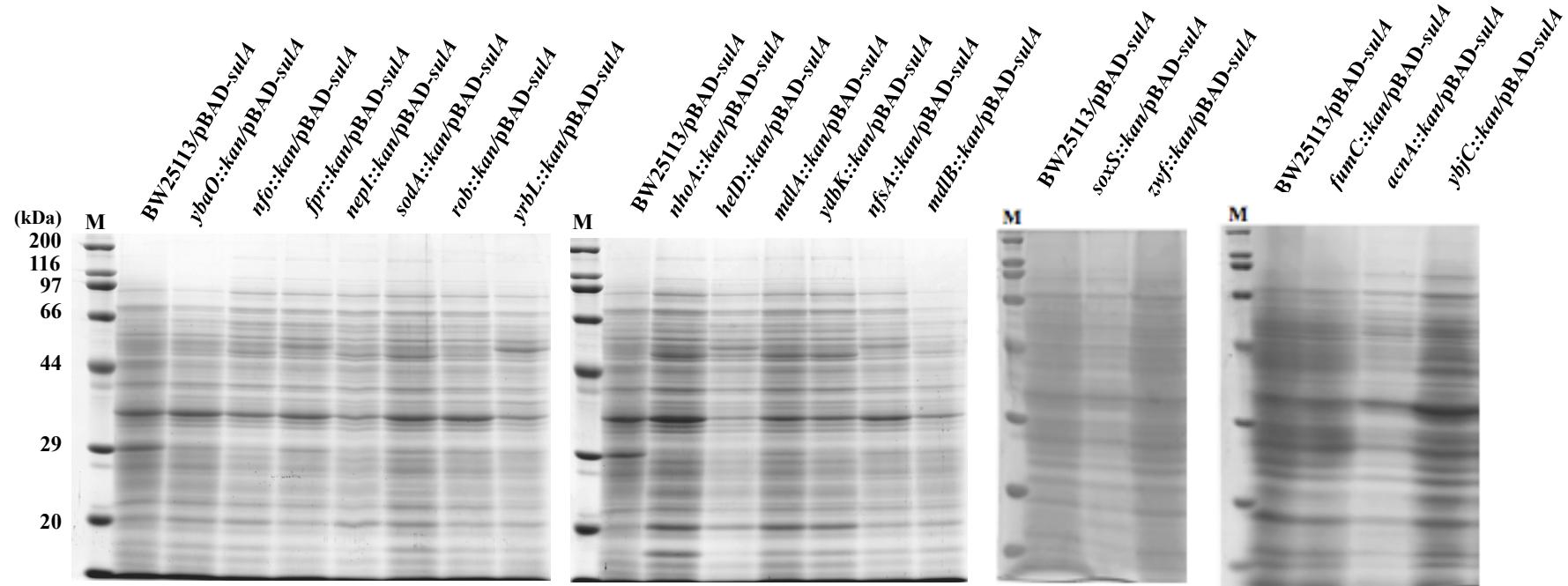


**Figure S3.** Effects of mitomycin C on cell growth and cell lysis. W3110N *Δlon sulA::kan* cells were grown in LB medium containing kanamycin, and mitomycin C (MMC) was added at 5 h (OD<sub>600</sub> of about 0.5) at the final concentrations of 0.1 mg/ml. Cell turbidity (straight lines) and colony forming unit (CFU) (dotted lines) were determined. Red circles and blue circles in (a) represent conditions with and without MMC, respectively. Proteins from the medium fraction were recovered and subjected to SDS-PAGE as described in Materials and methods. Lane M is molecular markers.



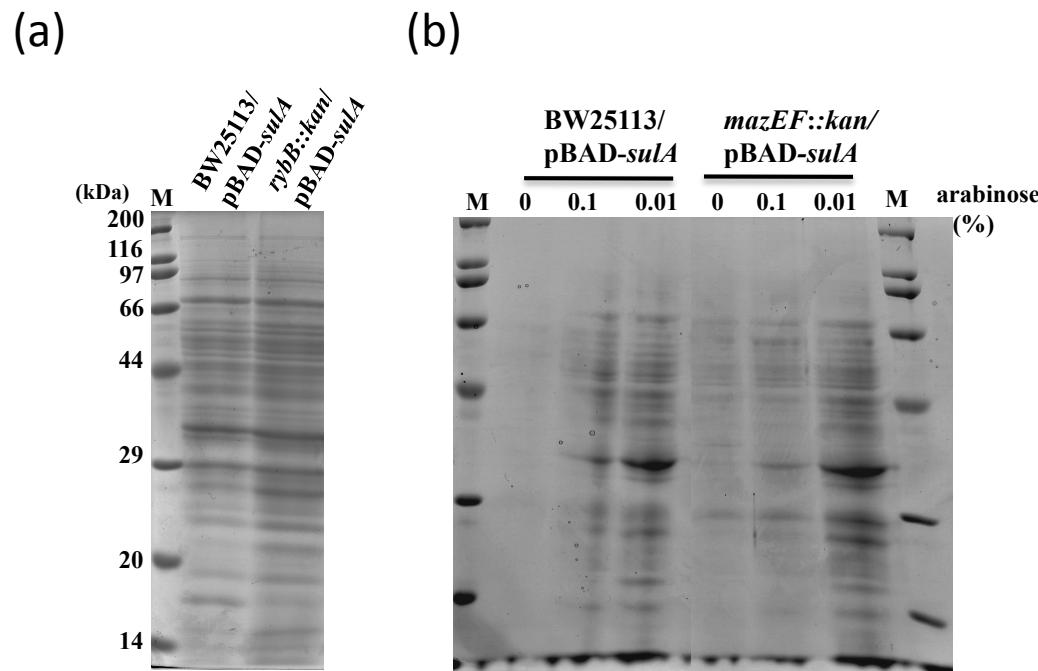
**Figure S4** Effect of disrupted mutation of *soxR* on SDCL.

*BW25113*, *BW25113 soxS::kan*, and *BW25113 soxR::kan* cells harboring *pBAD-sulA* were grown in LB medium containing ampicillin. L-Arabinose was added at OD<sub>600</sub> of about 0.5 at the final concentration of 0.1%. Portions of cultures were taken at 24 h after induction. Sample preparation for SDS-PAGE was performed as described in Materials and methods .



**Figure S5** Effects of disrupted mutation of 16 SoxS regulon genes on SDCL.

Gene-disrupted derivatives of BW25113 harboring pBAD-sulA and BW25113 harboring pBAD-sulA were grown in LB medium, and L-arabinose was added at OD<sub>600</sub> of about 0.5 at the final concentration of 0.01%. Portions of cultures were taken at 48 h after induction. Sample preparation for SDS-PAGE was performed as described in Materials and methods. BW25113 kan::helD and BW25113 kan::soxS were controls.



**Figure S6** Effects of *ΔrybB* and *ΔmazEF* on SDCL.

(a) BW25113 and BW25113 *rybB::kan* cells harboring pCA24N-*sulA* were grown in LB medium containing ampicillin. IPTG was added at OD<sub>600</sub> of about 0.5 at the final concentration of 0.1 mM. (b) BW25113 and BW25113 *mazEF::kan* cells harboring pBAD-*sulA* were grown in LB medium containing ampicillin. L-Arabinose was added at OD<sub>600</sub> of about 0.5 at the final concentration of 0.1% or 0.01%. Portions of cultures were taken at 24 h after induction. Sample preparation for SDS-PAGE was performed as described in Materials and methods.

**Table S1. Bacterial strains and plasmids**

Strains / plasmids	Relevant property	References
Strains		
W3110N	IN( <i>rrnD–rrnE</i> ) <i>rph-1</i> <i>rpoS</i> (33Q)	Kabir et al., 2004
BW25113	$\Delta$ <i>lacZ4787::rrnB-3 hsdR514</i>	Datsenko and Wanner
BW25113 <i>lon::kan</i>	BW25113 $\Delta$ <i>lon::kan</i>	Baba et al. (2006)
BW25113 <i>soxS::kan</i>	BW25113 $\Delta$ <i>soxS::kan</i>	Baba et al. (2006)
BW25113 <i>helD::kan</i>	BW25113 $\Delta$ <i>helD::kan</i>	Baba et al. (2006)
BW25113 <i>rybB::kan</i>	BW25113 $\Delta$ <i>rybB::kan</i>	Baba et al. (2006)
BW25113 <i>sodA::kan</i>	BW25113 $\Delta$ <i>sodA::kan</i>	Baba et al. (2006)
BW25113 <i>acnA::kan</i>	BW25113 $\Delta$ <i>acnA::kan</i>	Baba et al. (2006)
BW25113 <i>nhoA::kan</i>	BW25113 $\Delta$ <i>nhoA::kan</i>	Baba et al. (2006)
BW25113 <i>mdlA::kan</i>	BW25113 $\Delta$ <i>mdlA::kan</i>	Baba et al. (2006)
BW25113 <i>mdlB::kan</i>	BW25113 $\Delta$ <i>mdlB::kan</i>	Baba et al. (2006)
BW25113 <i>fpr::kan</i>	BW25113 $\Delta$ <i>fpr::kan</i>	Baba et al. (2006)
BW25113 <i>nfo::kan</i>	BW25113 $\Delta$ <i>nfo::kan</i>	Baba et al. (2006)
BW25113 <i>ybjC::kan</i>	BW25113 $\Delta$ <i>ybjC::kan</i>	Baba et al. (2006)
BW25113 <i>rob::kan</i>	BW25113 $\Delta$ <i>rob::kan</i>	Baba et al. (2006)
BW25113 <i>nepI::kan</i>	BW25113 $\Delta$ <i>nepI::kan</i>	Baba et al. (2006)
BW25113 <i>fumC::kan</i>	BW25113 $\Delta$ <i>fumC::kan</i>	Baba et al. (2006)
BW25113 <i>zwf::kan</i>	BW25113 $\Delta$ <i>zwf::kan</i>	Baba et al. (2006)
BW25113 <i>ybaO::kan</i>	BW25113 $\Delta$ <i>ybaO::kan</i>	Baba et al. (2006)
BW25113 <i>acnA::kan</i>	BW25113 $\Delta$ <i>acnA::kan</i>	Baba et al. (2006)
BW25113 <i>yrbL::kan</i>	BW25113 $\Delta$ <i>yrbL::kan</i>	Baba et al. (2006)
BW25113 <i>nfsA::kan</i>	BW25113 $\Delta$ <i>nfsA::kan</i>	Baba et al. (2006)
BW25113 <i>mazEF::kan</i>	BW25113 $\Delta$ <i>mazEF::kan</i>	This study
W3110N <i>lon::kan</i>	W3110N $\Delta$ <i>lon::kan</i>	This study
W3110N $\Delta$ <i>lon</i>	W3110N $\Delta$ <i>lon</i>	This study
W3110N $\Delta$ <i>lon sulA::kan</i>	W3110N $\Delta$ <i>lon</i> $\Delta$ <i>sulA::kan</i>	This study
Plasmids		
pBAD24	<i>araBAD</i> promoter, <i>amp</i> <sup>r</sup>	Guzman et al., (1995)

pBAD- <i>sulA</i>	pBAD24 bearing <i>sulA</i>	This study
pBAD- <i>soxS</i>	pBAD24 bearing <i>soxS</i>	This study
pBAD- <i>lpxC</i>	pBAD24 bearing <i>lpxC</i>	This study
pBAD- <i>held</i>	pBAD24 bearing <i>held</i>	This study
pCA24N	T-5 <i>lac</i> promoter, <i>lacI</i> <sup>q</sup> , <i>cml</i> <sup>r</sup>	Kitagawa <i>et al.</i> (2005)
pCA24N- <i>sulA</i>	pCA24N bearing <i>sulA</i>	This study
pCA24N- <i>soxS</i>	pCA24N bearing <i>soxS</i>	This study
pCA24N- <i>held</i>	pCA24N bearing <i>held</i>	Kitagawa <i>et al.</i> (2005)
pCA24N- <i>fldA</i>	pCA24N bearing <i>fldA</i>	Kitagawa <i>et al.</i> (2005)
pCA24N- <i>ribA</i>	pCA24N bearing <i>ribA</i>	Kitagawa <i>et al.</i> (2005)
pCA24N- <i>cspB</i>	pCA24N bearing <i>cspB</i>	Kitagawa <i>et al.</i> (2005)
pCA24N- <i>lpxC</i>	pCA24N bearing <i>lpxC</i>	Kitagawa <i>et al.</i> (2005)

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**Table 2S Primers used in this study**

Primer	Sequence
For construction of pCA24N recombinants	
F-soxS	GCCTCCCATCAGAAAATTATTCAAGGATCTTATCG
R-soxS	TTGCGGCCGCTTACAGGCGGTGGCGAT
F-sulA	GCCTACACTTCAGGCTATGCACATCG
R-sulA	TTGCGGCCGCTTAATGATAACAATTAGAGTGAATTAGCC
For construction of pBAD24 recombinants	
F-sulA	TTGAATTGGGGCTGGATTGATTATG
R-sulA	TTCTGCAGTCACGACTGAAAGCATT
F-soxS	TTGAATTACCATGTCCCCTCAGAAAATTATTCAAGG
R-soxS	TTAAGCTTTACAGGCGGTGGCGATAATC
F-lpxC	TTGAATTACCATGATCAAACAAAGGACACTAAC
R-lpxC	TTAAGCTTTATGCCAGTACAGCTGAAGGC
F- helD	TGCTAGCAGGAGGAATTACCATGGAACTGAAAGCGACAAC
R- helD	TTAAGCTTTACGGTTTCTGCCACCGGCACATCCAG
For confirmation of W3110N <i>lon::kan</i> or W3110N <i>Δlon sulA::kan</i> by PCR	
lon-F	GTCCGACAAAGCAAGCG
lon-R	CATCAGTAGATGCGCCC
For construction of W25113 <i>mazEF::kan</i>	
ΔmazEF-F	TTGATATATACTGTATCTACATATGATAGCGGTTGAGGAAAGG GTTATGGTGTAGGCTGGAGCTGCTTCG
ΔmazEF-R	GTGACCAGAATAGAAGTGAGTTAGTAACACTACCCAATCAGTA CGTTAATATTCCGGGGATCCGTCGACC
For confirmation of W25113 <i>mazEF::kan</i> by PCR	

AmazEF-U                    GTTGTACGTGATATCACGACCATT  
AmazEF-D                    TAAAACACCACCTGGAATAGCAGAT  
For RT-PCR  
sulA-F                    GCACATCGTTCTCGTC  
sulA-R                    AACCCCATAGCGTTACC

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**Table S3. Significantly up-regulated genes by overexpression of *sulA***

Gene	b-number	Ratio <sup>a</sup>	Function of product
<i>cspB</i>	b1557	5.141	Cold shock protein CspB
<i>cyoA</i>	b0432	3.719	Cytochrome <i>o</i> ubiquinol oxidase subunit I
<i>cyoB</i>	b0431	3.299	Cytochrome <i>o</i> ubiquinol oxidase subunit I
<i>cyoC</i>	b0430	3.948	Cytochrome <i>o</i> ubiquinol oxidase subunit III
<i>cyoD</i>	b0429	3.669	Cytochrome <i>o</i> ubiquinol oxidase subunit IV
<i>cyoE</i>	b0428	2.495	Protoheme IX farnesyltransferase (haeme O biosynthesis)
<i>fis</i>	b3261	2.291	Global DNA-binding transcriptional dual regulator
<i>flhC</i>	b1891	2.122	DNA-binding transcriptional regulator with FlhD
<i>fxsA</i>	b4140	2.214	Inner membrane protein
<i>gpp</i>	b3779	2.287	Guanosine pentaphosphatase/exopolyphosphatase
<i>held</i>	b0962	25.237	DNA Helicase IV (DNA repairing)
<i>infA</i>	b0884	2.552	Translation initiation factor IF-1
<i>lpdA</i>	b0116	2.182	Lipoamide dehydrogenase, E3 component is part of three enzyme complexes
<i>mukB</i>	b0924	2.390	Fused chromosome partitioning proteins
<i>mutT</i>	b0099	2.196	Nucleoside triphosphate pyrophosphohydrolase, marked preference for dGTP
<i>narG</i>	b1224	3.221	Nitrate reductase 1, alpha subunit
<i>narH</i>	b1225	2.718	Nitrate reductase 1, beta (Fe-S) subunit
<i>narJ</i>	b1226	2.282	Molybdenum-cofactor-assembly chaperone subunit (delta subunit) of nitrate reductase 1
<i>ndk</i>	b2518	2.178	Multifunctional nucleoside diphosphate kinase, apyrimidinic endonuclease, and 3'-phosphodiesterase
<i>nuoF</i>	b2284	2.412	NADH:ubiquinone oxidoreductase, chain F
<i>nuoG</i>	b2283	2.111	NADH-quinone oxidoreductase chain G
<i>nusA</i>	b3169	2.190	Transcription termination/antitermination L factor
<i>pstS</i>	b3728	2.181	Phosphate transporter subunit
<i>rho</i>	b3783	2.037	Transcription termination factor Rho
<i>rplB</i>	b3317	2.192	50S ribosomal protein L2
<i>rplC</i>	b3320	2.776	50S ribosomal protein L3

<i>rplM</i>	b3231	2.207	50S ribosomal protein L13
<i>rplQ</i>	b3294	2.190	50S ribosomal subunit protein L17
<i>rplV</i>	b3315	2.206	50S ribosomal subunit protein L22
<i>rplW</i>	b3318	2.357	50S ribosomal protein L23
<i>rpmA</i>	b3185	2.089	50S ribosomal protein L27
<i>rpmC</i>	b3312	2.155	50S ribosomal protein L29
<i>rpmD</i>	b3302	2.220	50S ribosomal subunit protein L30
<i>rpmI</i>	b1717	2.192	50S ribosomal subunit protein L35
<i>rpsC</i>	b3314	2.187	30S ribosomal subunit protein S3
<i>rpsF</i>	b4200	2.389	30S ribosomal subunit protein S6
<i>rpsI</i>	b3230	2.375	30S ribosomal subunit protein S9
<i>rpsJ</i>	b3321	2.154	30S ribosomal protein S10
<i>rpsK</i>	b3297	2.521	30S ribosomal protein S11
<i>rpsO</i>	b3165	2.215	30S ribosomal protein S15
<i>rpsS</i>	b3316	2.726	30S ribosomal protein S19
<i>sdiA</i>	b1916	2.144	DNA-binding transcriptional activator(transcriptional regulator of <i>ftsQAZ</i> gene cluster)
<i>secY</i>	b3300	2.502	SecYEG protein translocase auxillary subunit
<i>sodA</i>	b3908	2.308	Superoxide dismutase [Mn]
<i>soxS</i>	b4062	2.269	DNA-binding transcriptional dual regulator
<i>speD</i>	b0120	2.850	S-adenosylmethionine decarboxylase
<i>speE</i>	b0121	2.572	Spermidine synthase
<i>spr</i>	b2175	2.109	Predicted peptidase, outer membrane lipoprotein
<i>sulA</i>	b0958	152.0	SOS cell division inhibitor
<i>thrC</i>	b0004	3.916	Threonine synthase
<i>tpx</i>	b1324	2.305	Lipid hydroperoxide peroxidase
<i>yaaJ</i>	b0007	2.775	Putative transporter protein (sodium transport)
<i>yafK</i>	b0224	2.216	Hypothetical protein (DNA topological change)
<i>ycjX</i>	b1321	2.177	Conserved hypothetical protein with nucleoside triphosphate hydrolase domain
<i>ydbA</i>	b1405	2.538	Hypothetical protein YdbA fragment
<i>yejG</i>	b2181	2.470	Hypothetical protein

<i>yejL</i>	b2187	2.341	Hypothetical protein
<i>yfhL</i>	b2562	3.870	Predicted 4Fe-4S cluster-containing protein
<i>yhcN</i>	b3238	2.441	Hypothetical protein YhcN precursor
<i>yifB</i>	b3765	3.927	Predicted bifunctional protein, enzyme and transcriptional regulator
<i>ykgK</i>	b0294	2.094	Predicted regulator
<i>ylaC</i>	b0458	2.711	Predicted inner membrane protein
<i>yliH</i>	b0836	2.093	Hypothetical protein

<sup>a</sup> Expression ratios of more than 2 fold. Values are the average of ratios of the *sulA* overexpression sample to the control sample from at least two independent spots.

**Table S4. Significantly down-regulated genes by overexpression of *sulA***

Gene	b-number	Ratio <sup>a</sup>	Function of product
<i>aceA</i>	b4015	0.374	Isocytorate lyase, glyoxylate cycle
<i>appY</i>	b0564	0.441	DNA-binding transcriptional activator
<i>borD</i>	b0557	0.397	predicted lipoprotein
<i>elaB</i>	b2266	0.403	Hypothetical protein (cell growth and/or maintenance) Bifunctional GDP-fucose synthetase:
<i>fcl</i>	b2052	0.261	GDP-4-dehydro-6-deoxy-D-mannose epimerase and GDP-4-dehydro-6-L-deoxygalactose reductase
<i>feaR</i>	b1384	0.455	DNA-binding transcriptional regulator
<i>fecD</i>	b4288	0.369	Iron-dicitrate transporter subunit
<i>gadA</i>	b3517	0.160	Glutamate decarboxylase A, PLP-dependent
<i>gadB</i>	b1493	0.160	Glutamate decarboxylase B, PLP-dependent
<i>gadE</i>	b3512	0.303	DNA-binding transcriptional activator
<i>galU</i>	b1236	0.332	Glucose-1-phosphate uridylyltransferase
<i>glcG</i>	b2977	0.381	Hypothetical protein
<i>gmd</i>	b2053	0.337	GDP-D-mannose dehydratase, NAD(P)-binding
<i>intR</i>	b1345	0.388	Integrase
<i>kch</i>	b1250	0.487	Voltage-gated potassium channel
<i>malZ</i>	b0403	0.441	Maltodextrin glucosidase
<i>mhpF</i>	b0351	0.453	Acetaldehyde-CoA dehydrogenase II, NAD-binding
<i>msyB</i>	b1051	0.441	Hypothetical protein
<i>otsA</i>	b1896	0.314	Trehalose-6-phosphate synthase
<i>slp</i>	b3506	0.298	Outer membrane lipoprotein
<i>ugd</i>	b2028	0.257	UDP-glucose 6-dehydrogenase
<i>wcaA</i>	b2059	0.356	Predicted glycosyl transferase
<i>wcaB</i>	b2058	0.406	Predicted acyl transferase
<i>wcaF</i>	b2054	0.217	Predicted acyl transferase
<i>yaeR</i>	b0187	0.357	Predicted lyase
<i>yaiA</i>	b0389	0.369	Hypothetical protein
<i>ybgS</i>	b0753	0.173	Hypothetical protein

<i>ycaC</i>	b0897	0.385	Predicted hydrolase
<i>yceK</i>	b1050	0.421	Predicted lipoprotein
<i>ydeI</i>	b1536	0.321	Hypothetical protein
<i>ydhT</i>	b1669	0.457	Hypothetical protein
<i>yeaO</i>	b1792	0.375	Hypothetical protein
<i>yebQ</i>	b1828	0.463	Predicted transporter
<i>yecT</i>	b1877	0.371	Hypothetical protein
<i>yfaP</i>	b2225	0.312	hypothetical protein
<i>ygdI</i>	b2809	0.331	Hypothetical lipoprotein
<i>ygdR</i>	b2833	0.459	Hypothetical protein
<i>yghA</i>	b3003	0.346	Predicted glutathionylspermidine synthase, with NAD(P)-binding Rossmann-fold domain
<i>yhhH</i>	b3483	0.354	Hypothetical lipoprotein
<i>yiaB</i>	b3563	0.260	conserved inner membrane protein
<i>yigF</i>	b3817	0.181	Conserved inner membrane protein
<i>yigG</i>	b3818	0.281	Predicted inner membrane protein
<i>yjbD</i>	b4023	0.461	Hypothetical protein
<i>yjbE</i>	b4026	0.178	Hypothetical protein
<i>yjbF</i>	b4027	0.194	Predicted lipoprotein
<i>yjbJ</i>	b4045	0.431	Predicted stress response protein
<i>yjdJ</i>	b4127	0.367	Predicted acyltransferase with acyl-CoA N-acyltransferase domain
<i>ymfI</i>	b1143	0.323	Predicted DNA-binding transcriptional regulator
<i>yqeI</i>	b2847	0.388	Predicted transcriptional regulator
<i>yqeJ</i>	b2848	0.328	Hypothetical protein
<i>ytfE</i>	b4209	0.165	Predicted regulator of cell morphogenesis and cell wall metabolism

<sup>a</sup>Expression ratios of less than 0.5 fold. Values are the average of ratios of the *sulA* overexpression sample to the control sample from at least two independent spots.

**Table S5. Summary of significantly up-regulated and down-regulated genes by overexpression of *sulA***

Classification		Up-regulated gene <sup>a</sup>	Down-regulated gene <sup>b</sup>
A	Amino acid metabolism	<i>thrC</i>	<i>wcaB</i>
B	Cell envelope	<i>flhC, spr</i>	<i>ygdI</i>
C	Cellular process	<i>mukB, secY, sodA, tpx</i>	<i>msyB, otsA</i>
D	Central intermediary metabolism	<i>speD, speE</i>	<i>aceA, appY, fcl, gadA, gadB, glcG, gmd, ugd</i>
E	Energy metabolism	<i>cyoA, cyoB, cyoC, cyoD, cyoE, lpdA, narG, narH, narJ, nuoF, nuoG</i>	<i>galU, malZ, mhpF, wcaF</i>
F	Nucleotide metabolism	<i>mutT, ndk</i>	
G	Regulatory functions	<i>gpp, sdiA, soxS</i>	<i>fear, gadE</i>
H	Replication	<i>fis, held</i>	
I	Transport/binding protein	<i>pstS</i>	<i>fecD, kch</i>
J	Translation	<i>infA, rplB, rplC, rplM, rplQ, rplV, rplW, rpmA, rpmC, rpmD, rpmI, rpsC, rpsF, rpsI, rpsJ, rpsK, rpsO, rpsS</i>	
K	Transcription	<i>nusA, rho</i>	
L	Other categories	<i>cspB, fxsA</i>	<i>borD, intR, wcaA</i>
M	Hypothetical	<i>yaaJ, yafK, ycjX, ydbA, yejG, yejL, yfhL, yhcN, yifB, ykgK, ylaC, yliH</i>	<i>elaB, slp, yaeR, yaiA, ybgS, ycaC, yceK, ydeI, ydhT, yeaO, yebQ, yecT, yfaP, ygdR, yghA, yhhH, yiaB, yigF, yigG, yjbD, yjbE, yjbF, yjbJ, yjdJ, ymfI, yqeI, yqwJ, ytfE</i>
Total number of genes		62	51

<sup>a</sup> Up-regulated genes with expression ratios of more than 2 fold.

<sup>b</sup> Down-regulated genes with expression ratios of less than 0.5 fold.